DISSERTATION ON

PREDICTING THE IMPACT OF HIV INFECTION AND ANTIRETROVIRAL THERAPY ON THE DEVELOPMENT OF SUBCLINICAL ATHEROSCLEROSIS BY CAROTID INTIMAL MEDIAL THICKNESS

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CERTIFICATE

This is to certify that this dissertation entitled **"PREDICTING THE IMPACT OF HIV INFECTION AND ANTIRETROVIRAL THERAPY ON THE DEVELOPMENT OF SUBCLINICAL ATHEROSCLEROSIS BY CAROTID INTIMAL MEDIAL THICKNESS"** submitted by **Dr. MANOKARAN.S** appearing for Part II M.D. Branch I General Medicine Degree examination in April 2011 is a bonafide record of work done by him under my direct guidance and supervision in partial fulfillment of regulations of the Tamil Nadu Dr.M.G.R. Medical University, Chennai. I forward this to The Tamil Nadu Dr.M.G.R. Medical University, Chennai, Tamil Nadu, India.

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ABBREVIATIONS

ABCA-1	Adenosine triphosphate Binding Cassette Transporter-1
ACTG	AIDS Clinical Trial Group
ART	Antiretroviral therapy
BMI	Body Mass Index
CAD	Coronary Arterial Disease
CCL-2	Chemokine (C–C) motif ligand-2
CD	Cluster Differentiation
CHD	Coronary Heart Disease
CRP	C-Reactive Protein
CVD	Cardiovascular diseases
ECM	Extracellular matrix
FGF	Fibroblast Growth Factor
FRAM	Fat Redistribution And Metabolic changes in HIV infection
HAART	Highly Active Antiretroviral Treatment
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
hsCRP	high sensitivity CRP
IFN	Interferon

IL	Interleukin
IMT	Intimal Medial Thickness
LDL	Low Density Lipoprotein
MCP-1	Monocyte Chemotactic Protein-1
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial Infarction
mtDNA	mitochondrial Deoxyribonucleic acid
NACO	National AIDS Control Organization
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
PDGF	Platelet Derived Growth Factor
PI	Protease Inhibitor
PPAR	Peroxisome Proliferator Activated Receptor
TGF	Transforming Growth Factor
TNF	Tumor Necrosis Factor
UNAIDS	Joint United Nations Programme on HIV/AIDS
VCAM	Vascular Cell Adhesion Molecule
WHO	World Health Organisation

AIMS AND OBJECTIVES

HIV infection and the use of antiretroviral treatment may be a risk factor for premature atherosclerosis development, hence for increased cardiovascular and cerebrovascular morbidities and mortalities in these groups. Our aim was,

- To measure the carotid intimal-medial thickness in HIV infected subjects with and without antiretroviral therapy

To compare intimal-media thickness (IMT) of the carotid artery between HIV-infected subjects (without and with antiretroviral therapy) and controls.

INTRODUCTION

Human immunodeficiency virus (HIV) infection/Acquired Immunodeficiency Syndrome is a global pandemic. According to WHO-UNAIDS, 33.2 million individuals were living with HIV infection according to 2007statistics [(1)]. As per the provisional HIV estimate of 2008-09, there are an estimated 22.7 lakhs people living with HIV/AIDS in India. The HIV prevalence rate in the country is 0.29 percent (2008-09) [(2)].NACO launched Highly Active Anti Retroviral Therapy (HAART) on April 2004 in India. Currently there are 285 ART centres in India, and about 3.6 lakhs patients are on ART (august 2010) [(3)].

With the introduction of antiretroviral therapy (ART), the survival of HIV-infected individuals has dramatically improved and there are significant reduction in the HIV-related morbidity and mortality [(4, 5, 6)].

In the context of declining rates of HIV-related death, proportions of HIV infected patients dying of other causes have increased. In United States, the proportion of deaths among HIV-infected patients due to non–HIV-related causes increased from19.8% to 26.3% between 1999 and 2006 [(7)]. Cardiovascular and the cerebrovascular diseases were leading among the causes. However, it is controversial whether HIV infection contributes to accelerated atherosclerosis independent of traditional CVD risk factors.

So, there is an urgent need to determine whether people with HIV infection are at an increased risk for atherosclerosis. Various lipid dyscrasias are well

documented in HIV infected patients with ART therapy. In addition to lipid dyscrasias, patients with HIV infection also experience metabolic abnormalities including insulin resistance, visceral adiposity, and chronic immune activation. However, the relation between the metabolic abnormalities and increased risk of atherosclerosis is not well established.

FRAM (**F**at **R**edistribution **A**nd **M**etabolic Change in HIV Infection) study [8] shows that antiretroviral treatment and HIV infection contributes to accelerated atherosclerosis independent of traditional cardiovascular risk factors. But few other studies have varying results.

Measurement of carotid artery intimal-medial thickness (IMT) with high resolution B-mode ultrasound is a well-accepted, non-invasive method of assessing atherosclerosis and tracking its progression [9]. Carotid IMT measurements correlate well with pathological measurements and are potent predictors of myocardial infarction and stroke.

In our study, we evaluated the IMT of the carotid vessels to determine premature atherosclerotic lesions using an ultrasound colour-Doppler technique in HIV negative, HIV-infected subjects. This study was designed to identify the role of ART therapy and HIV infection on the risk of development of subclinical atherosclerosis by comparing the carotid intimal medial thickness.

REVIEW OF LITERATURE

Cardiovascular Complications Of HIV Infection And Antiretroviral Drugs:

As per the provisional HIV estimate of 2008-09, there are an estimated 22.7 lakhs people living with HIV/AIDS in India [(1)]. NACO launched Highly Active Anti Retroviral Therapy (HAART) on April 2004 in India. Currently there are 285 ART centres in India, and about 3.6 lakhs (16%) patients are on ART (august 2010) [(3)].

Epidemiology of Cardiovascular Disease in HIV-Infected Persons

In the context of declining rates of HIV-related death, proportions of HIV infected Patients dying of other causes have increased. For example, a death certificate study in New York City (USA) showed that the proportion of deaths among HIV-infected patients between 1999 to 2006, reflecting mortality resulting from non-HIV-related causes increased from19.8% to 26.3% mainly from cardiovascular disease (CVD), substance abuse, and non–AIDS-defining cancers [(4)]. Among individuals aged 55 years or older, CVD was the leading cause of death. Numerous studies have indicated the increased risk of myocardial infarction (MI) in HIV populations, with HIV infection considered at least a partial CVD risk factor in these studies. Klein and colleagues reported hospital-admission rates for coronary heart disease (CHD) in HIV-infected versus HIV uninfected populations of 6.5 versus3.8 per 1000 person-years [(10)] and 4.5 versus 2.9 per 1000 person-years in updated analyses with further follow-up time [(11)] respectively.

Figure 1 **Myocardial infarction rates among HIV infected versus HIV uninfected patients. Adopted from Triant et al (J of clin endocrinl metabol, 2007)**

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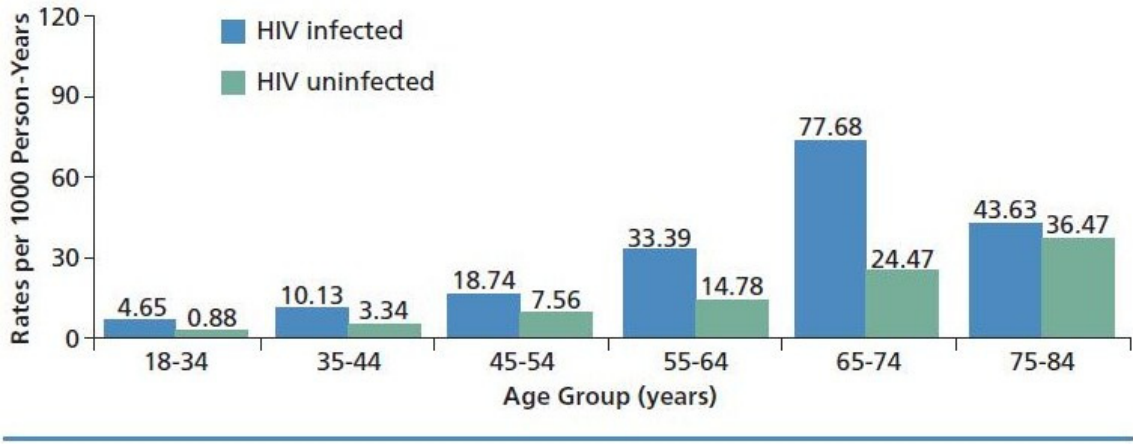


Currier and colleagues found a higher risk of coronary artery disease (CAD) admissions among younger HIV-infected than among HIV-uninfected patients[(12)]; Triant and colleagues found a 75% increase in risk of MI admissions in HIV-infected patients[(13)]; and Obel and colleagues found a 39% to 112% increased risk of CAD admissions in HIV-infected patients[(14)]. The study by Triant and colleagues was performed using data from a Massachusetts administrative hospital database including 3851 HIV-infected patients and more than 1 million HIVuninfected patients from 1996 to 2004.The mean MI rates were 11.13 versus 6.98 per 1000 person-years respectively. MI rates were higher in HIV-infected patients in all age groups, with very high rates in older patients. These investigators have also reported that levels of the acute-phase reactant C-reactive protein (CRP) were predictive of risk of MI in HIV-infected patients, despite the fact that CRP levels generally are non-specifically elevated

in HIV Infection [15]. These findings underscore the need to determine why the rates of CVD are higher in HIV-infected than –uninfected individuals. Understanding the relative contributions of host, virus, and antiretroviral therapy to risk of CVD in HIV infection will help in the development of strategies for prevention and treatment.

Pathophysiology Of Atherosclerosis In General: [16]

The mechanism of atherosclerosis is explained by the response to injury hypothesis. Endothelial cell injury is the corner stone of the response to-injury hypothesis. Endothelial loss due to any kind of injury—induced experimentally by mechanical denudation, hemodynamic forces, immune complex deposition, irradiation, or chemicals—results in intimal thickening; in the presence of high-lipid diets, typical atheromas ensue. However, early human lesions begin at sites of morphologically intact endothelium. Thus, endothelial dysfunction underlies human atherosclerosis; in this setting, dysfunctional endothelial cells show increased endothelial permeability, enhanced leukocyte adhesion, and altered gene expression.

The specific pathways and factors contributing to endothelial cell dysfunction in early atherosclerosis are not completely understood; etiologic culprits include hypertension, hyperlipidemia, and toxins from cigarette smoke. Inflammatory cytokines (e.g., tumour necrosis factor [TNF]) can also stimulate pro-atherogenic patterns of endothelial cell gene expression. However, the two

most important causes of endothelial dysfunction are hemodynamic disturbances and hypercholesterolemia.

Role Of Lipids In Atherosclerosis:

Lipids are typically transported in the bloodstream bound to specific apoproteins (forming lipoprotein complexes). Dyslipoproteinemias can result from mutations that alter the apoproteins or the lipoprotein receptors on cells, or from other disorders that affect the circulating levels of lipids (e.g., nephrotic syndrome, alcoholism, hypothyroidism, or diabetes mellitus). Common lipoprotein abnormalities in the general population (indeed, present in many myocardial infarction survivors) includes,

- Increased LDL cholesterol levels,
- Decreased HDL cholesterol levels, and
- Increased levels of the abnormal lipoprotein (a).

The mechanisms by which hyperlipidemia contributes to atherogenesis include the following:

Chronic hyperlipidemia, particularly hypercholesterolemia, can directly impair endothelial cell function by increasing local oxygen free radical production; oxygen free radicals can injure tissues and accelerate nitric oxide decay, reducing its vasodilator activity. With chronic hyperlipidemia, lipoproteins accumulate within the intima. These lipids are oxidized through the

action of oxygen free radicals locally generated by macrophages or endothelial cells. Oxidized LDL is ingested by macrophages through a scavenger receptor, distinct from the LDL receptor, and accumulates in phagocytes, which are then called foam cells. In addition, oxidized LDL stimulates the release of growth factors, cytokines, and chemokines by endothelial cells and macrophages that increase monocyte recruitment into lesions. Finally, oxidized LDL is cytotoxic to endothelial cells and smooth muscle cells and can induce endothelial cell dysfunction. The importance of oxidized LDL in atherogenesis is suggested by the fact that it accumulates within macrophages in all stages of plaque formation.

Monocytes transform into macrophages and avidly engulf lipoproteins including oxidized LDL. Monocyte recruitment and differentiation into macrophages (and ultimately into foam cells) is theoretically protective, because these cells remove potentially harmful lipid particles. However, the oxidized LDL augments macrophage activation and cytokine production (e.g., TNF). This further increases leukocyte adhesion and production of chemokines (e.g., MCP-1; monocyte chemotactic protein-1), creating a stimulus for recruitment of additional mononuclear inflammatory cells. Activated macrophages also produce reactive oxygen species that aggravate LDL oxidation and elaborate growth factors that drive smooth muscle cell proliferation.

T lymphocytes recruited to the intima interact with the macrophages and can generate a chronic inflammatory state. It is not clear whether the T cells are

responding to specific antigens (e.g., bacterial or viral antigens, heat-shock proteins, or modified arterial wall constituents and lipoproteins) or are non-specifically activated by the local inflammatory milieu. Nevertheless, activated T cells in the growing intimal lesions elaborate inflammatory cytokines (e.g., IFNγ) which can stimulate macrophages as well as endothelial cells and smooth muscle cells. As a consequence of the chronic inflammatory state, activated leukocytes and vascular wall cells release growth factors that promote smooth muscle cell proliferation and ECM synthesis.

Role Of Inflammation In Atherosclerosis:

Inflammatory cells and pathways contribute to the initiation, progression, and complications of atherosclerotic lesions. Although normal vessels do not bind inflammatory cells, early in atherogenesis, dysfunctional arterial endothelial cells express adhesion molecules that encourage leukocyte adhesion; vascular cell adhesion molecule 1 (VCAM-1), in particular, binds monocytes and T cells. After these cells adhere to the endothelium, they migrate into the intima under the influence of locally produced chemokines.

Smooth Muscle Proliferation:

Intimal smooth muscle cell proliferation and ECM deposition convert a fatty streak, the earliest lesion, into a mature atheroma and contribute to the progressive growth of atherosclerotic lesions (The intimal smooth muscle cells may be recruited from circulating precursors and they have a proliferative and synthetic phenotype distinct from the underlying medial smooth muscle cells).

Several growth factors are implicated in smooth muscle cell proliferation and ECM synthesis, including PDGF (released by locally adherent platelets, as well as macrophages, endothelial cells, and smooth muscle cells), FGF, and TGF-α. The recruited smooth muscle cells synthesize ECM (notably collagen) that stabilizes atherosclerotic plaques. However, activated inflammatory cells in atheromas can cause intimal smooth muscle cell apoptosis, and also increase ECM catabolism resulting in unstable plaques.

Factors Behind The Accelerated Atherosclerosis In HIV Patients:

Host Factors

Traditional CVD risk factors, as well as HIV infection and its treatment, contribute to the risk of CVD in HIV-infected individuals. The risk of MI in both HIV-infected and -uninfected populations is increased in a similar manner by the risk factors of increasing age, male sex, diabetes, smoking, and hypertension[17]. The prevalence of some risk factors may be higher in HIV-infected populations. A recent analysis of modifiable risk factors and death in the D: A: D (Data Collection on Adverse Events of Anti-HIV Drugs) study [18] showed that smoking (rate ratio, 1.20), hypertension (rate ratio, 1.53), and diabetes (rate ratio, 1.83) were independently associated with risk of death during treatment for HIV infection [19]. Smoking is common in many HIV-infected populations, and programs aimed at cessation have thus far been largely unsuccessful [20].

Greater attention needs to be given to smoking and other modifiable CVD risk factors in HIVinfected patients. Increased carotid artery intima-media thickness (IMT), a marker for subclinical atherosclerosis, is associated with increased risk of MI. An analysis from the FRAM (Fat Redistribution And Metabolism) study showed that HIV infection was associated with statistically significant increase in internal carotid (0.15 mm; $P < .001$) and common carotid

(0.033 mm; $P < .01$) IMT compared with IMT values for a large population of HIV-uninfected persons[21]. Because most HIV infected patients in the FRAM study were receiving antiretroviral therapy, any potential effect of anti retroviral therapy on this finding is uncertain. Other traditional risk factors associate with increased IMT in the FRAM patients were male sex, current and past smoking, diabetes, age per 10-year increase, systolic blood pressure increase, and total cholesterol level increase. High-density lipoprotein (HDL) cholesterol level increase was associated with a statistically significant reduction in IMT.

Virus Factors

The SMART (Strategies for Management of Antiretroviral Therapy) study examined the potential for reducing antiretroviral therapy toxicity by limiting time on treatment, with 5472 patients with CD4+ counts higher than 350 cells/μL randomly assigned to a treatment-interruption plan (termed drug-conservation strategy) or to continuous treatment (viral-suppression strategy). The drug-conservation strategy increased risk over the viral suppression strategy of opportunistic disease or death (rates, 3.4% vs1.3%, respectively; hazard ratio [HR],2.6) and of CVD, renal, or liver events(rates, 1.8% vs 1.1%, respectively; HR,1.7) [22]. The study brought into focus the importance of serious non–AIDS-related events among patients without viral suppression on antiretroviral therapy. Subsequent analysis did not find an association between

viral load and risk of CVD, but confirmed increased risk of CVD among patients who discontinued antiretroviral therapy.

A number of mechanisms have been proposed to explain how HIV infection might contribute to atherosclerosis.

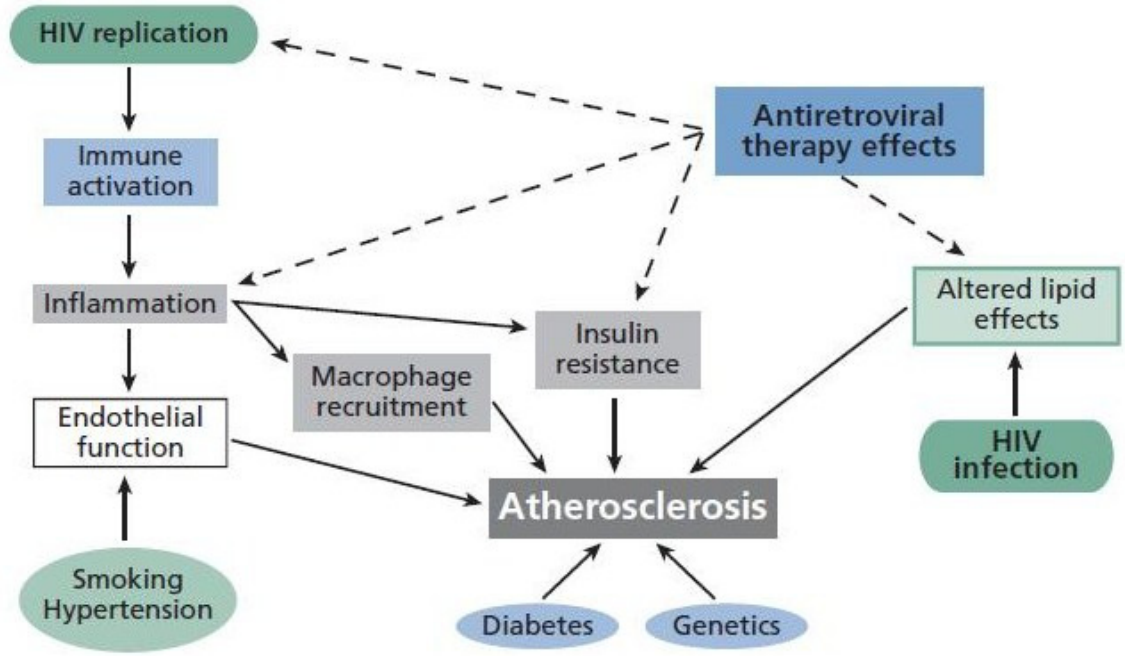
1. HIV has been reported to infect smooth muscle cells in vitro and in vivo.
2. HIV increases secretion of Monocyte chemo attractant molecules like CCL2, and MCP-1. This facilitates development of foam cells [(23)].
3. Macrophages, which play a pivotal role in atherosclerosis, are also hosts for HIV.
4. It is proved that there is impairment of cholesterol efflux (reverse cholesterol transport) in HIV-infected macrophages. HIV Nef protein is critical for cholesterol efflux impairment. It specifically targets adenosine triphosphate binding cassette (ABCA-1) transporter -dependent cholesterol efflux and also down-regulates ABCA1. Nef alters intracellular distribution of ABCA1. HIV-infected macrophages transform into foam cells and they are found in atherosclerotic plaques of HIV-infected patients. Active cholesterol efflux reduces infectivity of HIV virions. These pathological effects of HIV may initiate and accelerate the plaque formation in vessel walls [(24)].
5. HIV tat protein interaction with signal transduction pathways that lead to increased expression of vascular endothelial growth factors and platelet

activating factor; and HIV membrane micro particles inhibit the endothelial nitric oxide synthase expression.

6. This effect of Nef inhibition on the ABCA-1transporter has been demonstrated in simian immunodeficiency virus (SIV)-infected macaques[25].
7. HIV may also directly impair HDL metabolism, thus enhancing transfer of HDL to atherogenic apolipoprotein B lipoproteins [26]. This potential mechanism is consistent with findings in the recent SMART trial analysis indicating an association of total HDL particles with risk of CVD in patients in the drug-conservation group [27]. In particular, risk was elevated in patients with declining HDL cholesterol levels

Collectively, these findings suggest that untreated HIV infection could contribute to the development of atherosclerosis, although the magnitude of the effects remains unclear.

Figure 2 **Interaction of host, virus, and antiretroviral therapy effects in cardiovascular disease risk**



Another mechanism by which HIV infection itself may contribute to CVD risk is inflammation. CRP is a marker of inflammation that independently predicts risk of CVD in adults in the general population. In HIV infection, elevated CRP levels predict HIV disease progression and mortality in untreated HIV patients after adjustment for viral load and CD4+ count. Uncontrolled HIV infection is associated with elevated markers of inflammation, including CRP. Levels of these markers decline with treatment but not to normal levels. Little is known about how different antiretroviral drugs affect CRP levels during successful anti retroviral therapy. Recent data from ACTG (AIDS Clinical Trials Group) study 5095 demonstrated that CRP levels did not improve during 96 weeks of treatment with efavirenz; in fact, among women CRP levels rose [(28)]. Elevated baseline levels of the inflammatory marker interleukin-6 (IL-6)

and the coagulation marker D-dimer were associated with all-cause mortality (not specifically with CVD events) in the SMART trial, and levels of these markers rose after treatment interruption [29].Despite the potential association of inflammation with increased CVD risk, a number of small studies have not found a strong association between higher levels of high-sensitivity CRP (hsCRP) and IMT. Hsue and colleagues found no association of hsCRP or immune activation (CD38+, CD4+, CD8+ cell responses) with IMT, but they reported an association between IMT and cytomegalovirus-specific T-cell responses, suggesting that response to latent or persistent viral infection might be driving a pro-atherosclerotic response [30]. Other findings include improved endothelial function (measured by brachial artery reactivity) after 24 weeks of antiretroviral therapy in treatment-naïve patients but no significant change in hsCRP level. In a pilot study in patients with untreated HIV infection, 8 weeks of treatment with the tumour necrosis factor inhibitor pentoxifylline resulted in improvements in the endothelial activation marker VCAM-1 and brachial artery flow-mediated dilation[31].The effects of such an approach to reducing inflammation in antiretroviral therapy–treated patients with viral suppression are being investigated.

Antiretroviral Therapy Factors

Recent studies showed that the risk of MI increases by 26% per year of HAART in HIV patients when compared to the non-HIV patients.

Endothelial Dysfunction In ART Therapy

Endothelial dysfunction, a likely precursor of early atherosclerosis, has been reported in HAART-naive patients and in patients treated with PIs and nucleoside reverse transcriptase inhibitors (NRTIs). Similarly, HAART-mediated endothelial dysfunction has been attributed to a reduction in nitric oxide production or release[32], and an increase in reactive oxygen species production [33, 34], as well as impairment of cholesterol efflux and accelerated foam cell formation [35, 36].

Lipid And Metabolic Abnormalities In ART Therapy

The etiology and development of the dyslipidaemic, anthropometric and metabolic changes observed in chronically HIV-infected patients and its interplay with enhanced CVD risk is complex [37]. These changes often coexist and are modulated by therapy with HAART, genetic and dietary factors and together appear to exert synergistic atherogenic effects on the vasculature.

Lipid abnormalities are characterised by low levels of high-density lipoprotein (HDL) and LDL cholesterol and high levels of very low-density lipoprotein (VLDL) and triglycerides [37, 38]. Overall, this lipid milieu may be atherogenic due to low HDL levels and an increase in de novo lipogenesis and reduced VLDL clearance [39]. Various PIs, in particular ritonavir, can cause hypertriglyceridaemia [40] while other agents increase LDL modestly [41]. Carr et

al hypothesised that the catalytic region of HIV-1 protease (the binding site of various PIs) possesses some homology to regions of proteins regulating lipid metabolism (cytoplasmic retinoic acid-binding protein-I and LDL receptor-related protein) so that PI binding to these proteins results in impaired hepatic chylomicron uptake and triglyceride clearance by LDL receptor-related protein-lipoprotein-lipase complex [42, 43]. Other mechanisms of PI-induced dyslipidaemia have been described and include inhibition of Apo-B degradation [44].

With the advent of HAART, anthropometric changes in HAART-naive patients were observed following NRTI and PI treatment, which were characterised by early increases in subcutaneous and visceral fat followed by loss of subcutaneous fat (peripheral lipoatrophy) with relative preservation of visceral fat (visceral adiposity) [42, 45, 46]. Such lipodystrophic changes have been reported in 30-50% of HAART users (particularly with stavudine and didanosine or zidovudine and lamivudine combined with nelfinavir, efavirenz, or both) [47, 48] while up to 26% of HIV-infected patients can be diagnosed with metabolic syndrome [49, 50]. The pathogenesis of HAART-induced lipodystrophic changes is complex [37] and may involve mitochondrial toxicity [51] and modulation of adipocyte differentiation [52]. Visceral fat accumulation is a pro-inflammatory state because visceral adipose tissue produces and secretes proinflammatory cytokines (TNF-[alpha], IL-6, MCP-1) and adipokines

(adiponectin, leptin and resistin) that attract and recruit macrophages to adipose tissue, stimulate hepatic CRP production and exacerbate insulin resistance [49,53]. Once activated, macrophages elaborate further proinflammatory mediators continually driving chronic vascular inflammation [49].

Metabolic changes, in particular insulin resistance, may result from the developing anthropometric changes as well as from the direct effects of HAART[54]. Diabetes mellitus has been reported to occur in 6-18% of HIV-infected patients[54] whilst the prevalence of insulin resistance may be as high as 50% in those with anthropometric abnormalities [55]. PIs and NRTIs have been most frequently implicated via selectively inhibiting the transport function of Glut 4 receptor [56] and inhibiting mitochondrial function in skeletal muscles [57], respectively.

Cholesterol Efflux Impairment In HIV Patients

Accumulation of oxidised LDL cholesterol in macrophages due to unregulated uptake by CD36 scavenger receptors leads to foam cell formation. There is evidence that PIs can upregulate the macrophage CD36 scavenger receptors to promote cholesterol ester accumulation [35, 36, 49].

In the current HAART era, PI-based regimens are common and have been associated with elevations in VLDL and LDL levels [58-60], which may translate into greater clinical risk of CVD. More recently, we have shown that HIV itself, rather than the commonly used antiretroviral compounds (stavudine, efavirenz, nevirapine, lopinavir, amprenavir, nelfinavir and ritonavir), impairs cholesterol efflux [61]. This implies that HAART promotes atherosclerosis mainly by enhancing forward cholesterol transport and delivery of LDL and oxidised LDL to macrophages, while HIV appears responsible for the inhibition of reverse cholesterol transport.

Protease inhibitors (PIs) may influence lipid metabolism by interfering with the degradation by proteasomes in hepatocytes and adipocytes, thus influencing the expression of genes involved in lipid metabolism. Genes involved in lipid synthesis were also upregulated in hepatocytes by lopinavir, ritonavir, and nelfinavir, but repressed in adipocytes, consistent with the observations in vivo of increased hepatic lipid synthesis but reduced storage in fat cells. Inhibition of proteasomes in vivo with the resultant impact on lipid biosynthesis may be the etiology of PI-associated dyslipidaemia, and the

absence of an effect of atazanavir on these pathways may explain the absence of dyslipidaemia seen with this drug in vitro [62].

There is also great interest in the role of adiponectin, a hormone secreted by adipocytes, in the pathogenesis of body composition changes observed during treatment of HIV infection. Cross-sectional studies have demonstrated reduced circulating levels of adiponectin in patients with both lipoatrophy and lipohypertrophy [62]. In HIV patients, the mitochondrial and peroxisome proliferator-activated receptor-γ (PPAR–γ) gene expression decreased significantly [62]. Among those on antiretroviral therapy, use of a non nucleoside reverse transcriptase inhibitor (NNRTI) was associated with higher HDL-c [63].

Nucleoside reverse transcriptase inhibitors (NRTIs) act via their incorporation into the growing viral DNA chain during reverse transcription, with incorporation resulting in chain termination. The NRTIs also inhibit gamma polymerase in vitro, and thus inhibit mtDNA synthesis. Consequent mtDNA depletion (or mutation) can result in insufficient energy production and cell dysfunction and in tissue and organ dysfunction when sufficient numbers of normally functioning mitochondria are not present [64]. In addition to the potential effects of gamma polymerase inhibition, NRTIs may also be associated with oxidative damage to mitochondria [65], inhibition of mitochondrial enzymes [66], uncoupling of the electron transport chain from ATP (Adenosine triphosphate) synthesis, and induction of apoptosis[67].

Complications of NRTIs include cardiomyopathy, myopathy, peripheral neuropathy, pancreatitis, proximal renal tubular dysfunction, and hepatic steatosis and lactic acidosis. Many of the effects associated with mitochondrial toxicity are difficult to distinguish from effects associated with HIV infection itself. The phenotype of lipoatrophy in HIVinfected patients receiving antiretroviral therapy at least superficially resembles that in Madelung's disease (multiple symmetric lipomatosis), which is associated with mtDNA mutations. It has been hypothesized that mitochondrial toxicity of adipocytes associated with antiretroviral therapy may lead to adipocyte apoptosis and thus lipoatrophy [68, 69]. Some support for this hypothesis is provided by the following findings from studies in patients receiving therapy: an association of lipoatrophy with hyperlactatemia and liver dysfunction in a patient series [70]; abnormal adipocyte mitochondria on fat biopsies in a small number of patients with lipoatrophy [71]; a 60% frequency of decreased mtDNA levels in subcutaneous fat biopsies (neck, abdomen, thigh) in patients with lipodystrophy versus a frequency of 0% to 30% in patients without lipodystrophy or in HIV-seronegative subjects [72]; and the association of NRTI use with a decrease in mtDNA (mean, 44%) in fat biopsies in buttocks [73]. In conclusion, accelerated atherosclerosis in ART therapy is multifactorial.

LIMITATIONS OF THE STUDY

- Limitations of our study include the use of cross-sectional data for both risk factors and cIMT. Given that the effects of HIV and antiretroviral therapy on CVD risk factors are dynamic and cumulative, it might have been ideal to have longitudinal data for risk factors.
- Sample size is smaller.
- Findings of this study cannot be extrapolated outside the adult population because our subjects were aged from 30 to 46 years.
- We have also not included the post menopausal women in this study, so the influence of the post-menopausal state was left untouched.

DISCUSSION

We have shown that preclinical atherosclerosis as quantified by carotid Doppler ultrasound measurements of carotid IMT is increased in HIV-infected participants compared with controls, even after adjusting for demographic profiles and traditional CVD risk factors (gender, age, smoking, diabetes, and hypertension). This result correlate with the cross sectional study done by Grunfeld et al[75]. In that study 1183 HIV infected participants and 297 HIV uninfected controls (from the FRAM study and MESA study) were studied from 2000 to 2002. This study clearly codes that association between HIV infection and IMT was similar to that of the traditional risk factors like smoking.

In the total of 182 individuals studied, HIV naïve, as well as the HIV positives on ART both had higher level of carotid IMT when comparing to matched control group. The three groups were well matched in the demographic profiles and traditional cardiovascular risk factors. There were no differences in the waist circumferences, waist/hip ratio. The control group had higher BMI values when compared to HIV positives (group 2 & 3). This is in contrast to the study done by Maggi et al [76], in which the BMI values and waist circumference were higher in the individuals on ART therapy. But Maggi et al study had patients, who are on long duration protease inhibitor therapy and significant body fat redistributions.

The three groups had no differences in the total cholesterol levels, LDL cholesterol levels, HDL cholesterol levels. But M. Bongiovanni et al[77] study

showed that the total cholesterol levels were lower in the control group when compared to HIV seropositive groups. Among the seropositive individuals, HIV patients on HAART therapy had significantly higher total cholesterol levels than HIV naïve patients. Rose et al [78] showed that the HDL levels are lower in the HIV affected patient. But in this study, there are no differences in the HDL levels between the groups. This can be explained by the patients on ART therapy in this study were on non protease inhibitor based regimen (zidovudine + lamivudine and nevirapine). The NNRTI- nevirapine is the one drug which is shown to increase the HDL levels as described by Franssen et al [79].

HIV seropositive groups had higher levels of triglycerides, which was more in the HIV naïve patients than who are on ART. This results correlate with the results from the Grunfeld et al study [80]. This shows that HIV infection per se has effects on lipid metabolism in addition to chronic inflammation secondary to HIV, and due to ART therapy.

In this study distribution of smokers were equal in all the three groups. But in the Massachusetts hospitals databases the prevalence of the smoking and alcoholism were high in the HIV affected individuals. Smokers had higher carotid IMT levels than non-smokers. Among the HIV infected individuals, the smokers had higher carotid IMT than non smoker HIV seropositives. Atheromatous plaque was identified in 16 individuals. There were no differences in the distribution of atheromatous plaques between the groups but

the smokers had higher prevalence of plaques, which was statistically significant.

Carotid IMT values were higher in males than females. This results correlates with the study by Alessandra vigano et al in which male adolescents and young adults had higher carotid IMT values than matched females [(81)]. This may simply reflect the tendency of males to have higher values of IMT than females as described by Lee et al [(82)]. In the study done by Grunfeld et al [(83)], the HIV association with IMT was somewhat stronger among women than in men (HIV by gender interactions for men $p = 0.046$ and for women $p = 0.003$). But in our study the HIV association with IMT was equal in males and females (p value for males & females were <0.001). In our study we found that the CD4 count had negative correlation with the carotid IMT values. In a study done by Priscilla et al [(84)], they identified that the CD4 count had inverse relationship with the carotid IMT levels. The CD4 count has inverse relation with the HIV viral load. Drop in CD4 count indirectly indicates the increasing viral load and hence the disease progression. So the increased inflammation and the HIV virus itself (by infecting the vascular smooth muscles, acceleration of foam cell formation, and impaired cholesterol efflux from the macrophages) could contribute in the accelerated atherosclerosis. These effects of inflammation and virus in the accelerated atherosclerosis were studied by Mujawar et al [(85)] and Bukrinsky et al [(86)].

The HDL cholesterol levels also had inverse relationship with the carotid IMT values in HIV patients. This results correlates with the Rose et al study [87], in their study they proposed that the HIV may directly impair the HDL metabolism by enhancing the transfer of HDL to ApoB lipoprotein. Duprez et al [88] described that the decreasing HDL levels in HIV patients associated with increasing risk for cardiovascular diseases.

We did the correlation analysis of predictive factors versus the carotid IMT. The results showed that the age, gender, waist circumference, triglyceride levels, LDL cholesterol levels and the duration of the disease were shown to have direct correlation with the carotid IMT levels. Of these, the age and gender had the strongest impact over the carotid IMT values. In this study, it is shown that the HIV status has more impact than the smoking status on the carotid IMT values. HIV naïve patients and HIV seropositives on ART therapy had no significant difference in the triglyceride levels or in carotid IMT values. But in the studies done by currier et al [89], and jerico et al [90], patients on ART therapy had significant increase in the cholesterol, triglyceride levels and also in IMT values. In contrary to this, the duration of ART therapy was negatively correlated with the IMT values of our study. The probable explanation is, we took only the patients who are on two NRTIs (zidovudine and lamivudine) and one NNRTI (nevirapine) regimen. Moreover in Currier et al and Jerico et al studies, most of the patients were on protease inhibitors containing regimen and

also they were not on NNRTI; and the treatment duration was significantly higher than our study.

After multivariate regression adjustment for demographic characteristics (age, gender), the adjusted mean difference of HIV-infected vs controls was +0.04 mm (p <0.0001). Further adjustment for traditional CVD risk factors (smoking, elevated triglycerides, BMI, total cholesterol, LDL and HDL cholesterol) modestly attenuated the HIV effect (+0.036 mm, p<0.001).This shows that, the HIV infection was independently associated with greater IMT. The association of HIV infection with IMT was more than that of traditional risk factors such as smoking which was associated with greater IMT (+0.011 mm, p<0.001). Moreover the duration of the disease positively correlated to the higher IMT values. This is supported by the study done by the study done by Mercie et al [91].

MATERIALS AND METHODS

Settings

ART CENTRE,

Institute of internal medicine and Barnard institute of radiology,

Madras Medical College and Government General Hospital,

Chennai - 600 003.

Ethical Approval

Obtained

Study Duration

This study was conducted for a period of eighteen months from January 2009 to June 2010.

Study Design

To predict the impact of HIV infection and antiretroviral therapy on the development of subclinical atherosclerosis by carotid intimal medial thickness, a cross sectional design with 121 HIV-positive subjects and 61 age and sex-matched controls.

Inclusion Criteria

1. Patients with HIV infection and not on any antiretroviral therapy (Duration of disease > 2 years).
2. Patients with HIV infection and on antiretroviral therapy with the duration of >than 2 years (ZIDOVUDINE + LAMIVUDINE + NEVIRAPINE regimen).
3. Normal HIV unaffected individuals as controls

Exclusion Criteria

1. Patients with diabetes mellitus or current use of oral hypoglycaemic agents and/or Thiazolidinediones,
2. History of coronary heart disease or stroke.
3. Hypertension, endocrine disorders.
4. Family history of coronary heart diseases, stroke.Hypercholesterolemia.
5. Obesity [defined as a body mass index (BMI) > 30].
6. Subjects with abnormal renal or liver parameters [creatinine >1.6mg/dl; alanine aminotransferase or aspartate aminotransferase > 2.5 × upper limit of normal (ULN)].
7. Subjects requiring systemic chemotherapy, radiation therapy or systemic Steroids were excluded.
8. Patients having active opportunistic infections.
9. Patients with HBV and HCV infections.
10. Patients with CD4 count less than 250.

11. Patients with poor adherence to therapy.
12. Female patients with post menopausal state.
13. Patients on statins or anti-inflammatory drug therapy

Laboratory Methods

All patients with HIV referred to ART centre were subjected to a detailed clinical history regarding their age, occupation, socio economic status, educational status, possible route of acquisition of the disease, duration of disease, duration of ART(if any), marital status, history of opportunistic infections, sexually transmitted infections, previous HIV testing, medication/drug use, co morbidities like diabetes, hypertension, hepatitis B/C, history of vaccination, allergy, history regarding diet, smoking/alcoholism and family history regarding CVD risk factors. A complete physical examination was performed, including the measurement of blood pressure (determined by using a sphygmomanometer with the subjects in a sitting position after >30 min at rest) and the calculation of body mass index (BMI), Waist/Hip ratio.

Initial investigations included complete blood count, renal function tests, liver function tests, CD4 count. Blood was taken in a fasting state (>8 hrs of fasting) to test for blood sugar, total cholesterol, serum triglycerides, HDL-cholesterol, LDL-cholesterol concentration was calculated by the Friedewald formula [(74)]. CD4+ cell counts using the PARTEC flow cytometer (Germany).

All subjects underwent an ultrasonography of the carotid vessels using the ESAOTE (my lab 60) colour-Doppler with 7.5 MHz (LA 523) high frequency

probes. Characteristics of the intima, pulsation index, resistance index, minimal speed, peak speed and mean speed were evaluated. All the ultrasonographies were performed by the same sonologist, who was unaware of the HIV status, with the same colour power Doppler. Patients were submitted to the investigation in a supine position after at least 10 min of acclimatization in a comfortable room. During the examination, the head of the patient was extra-rotated from the opposite side. Once the image had been optimized, the bifurcations of the vessels were located and the echocardiograph zoom was deployed. The common carotid, the bifurcation and at least the first 2 cm of the internal and external carotid vessels were examined in the short and long axis during the tele-diastolic phase (T wave of the electrocardiogram). Carotid IMT was measured between the bifurcation and 1 cm proximal to the bifurcation (near the bulb). The same procedures were then repeated for the opposite side carotid vessels. Then the averages of two measurements were taken IMT as final reading.

METHODS

Study subjects are divided into three groups: group 1, group 2, and group 3.

Group 1

HIV-uninfected control subjects. They were selected from the patient's relatives from the internal medicine wards.

Group 2

HIV-infected subjects, who are having the disease more than 2 years. Not yet started on ART therapy (defined as not receiving any regimen for more than a total of 3 months at any time prior to study entry).

Group 3

HIV-infected subjects who were currently receiving antiretroviral therapy (ZIDOVUDINE + LAMIVUDINE + NEVIRAPINE regimen). The subjects must have taken the treatment at least 2 years or more, continuous use prior to study. Interruptions in therapy for at most 4 weeks for management of toxicity was allowed.

Subjects from each group, were matched on five cardiovascular disease risk factors: age (within 5 years), sex, and smoking status (yes or no), and menopausal status. The matched design was employed in order to control for important traditional risk factors for atherosclerosis while attempting to isolate the effects of HIV infection and ART therapy on carotid IMT.

Study Population:

A total of 121 patients were enrolled for the study from the population of HIV infected patients who attended the ART clinic, Institute of Internal Medicine, from the period between January 2009 and June 2010. In which 64 were HIV naïve patients and remaining 57 are HIV patients on ART therapy.

Controls (61 volunteers) for the study were selected from the internal medicine wards, who came with the patients. Only who satisfied all the inclusion and exclusion criteria, were included for the study. Informed written consent was obtained from all patients participating in the study.

Statistical Analysis:

Data analysis was done with use of SPSS, version 15. Descriptive statistics were used to calculate the frequency, mean, median, and standard deviation. To examine the linear trend of the proportions, trend chi-square was used and to find the test of association, chi-square was computed. For all normally distributed variables, Student's *t* test was used to determine the significant mean difference in various groups. Multivariate regression analyses were performed to find potential predictors of carotid intimal thickening. The covariates considered for all subjects were: gender, age, smoking habit, BMI, waist circumference, waist hip ratio, triglycerides, total cholesterol, LDL, HDL. For the HIV-infected subjects, additional covariates included were duration of the disease, length of ART use (in months) and CD4 cell count ($\times 10^6$ cells/l). The results of the univariate analysis were used as a guide for the multivariate analysis. The primary hypothesis was to investigate the difference in carotid IMT between groups. The planned comparison of group 1 to group 2+3 was to assess the effect of HIV infection on carotid IMT. The planned comparison of group 2with group 3 was to assess the effect of ART therapy on carotid IMT in the HIV-infected subjects.

OBSERVATION AND RESULTS

A total of 182 patients were studied. In which, controls were taken as group 1, HIV naive patients were taken as group 2, HIV patients who are on ART were considered as group 3. Females were 65(35.7%). Mean age was 38 years (30-46). For both age and sex, there were no differences between groups ($p>0.05$). The details of the age and sex distribution among groups are given below.

Figure 1 **Percentage of individuals in each group.**

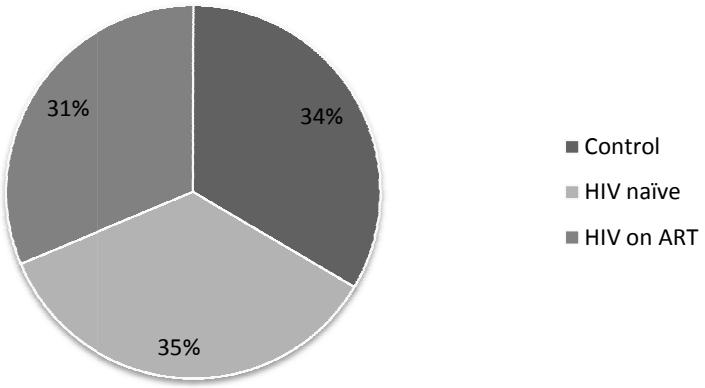


Table 1 **Percentage of individuals in each group.**

Groups	No. of individuals	Percentage (%)
Control	61	33.5
HIV without ART	64	35.2
HIV with ART	57	31.3
Total	182	100.0

Figure 2 **Age distribution among the groups.**

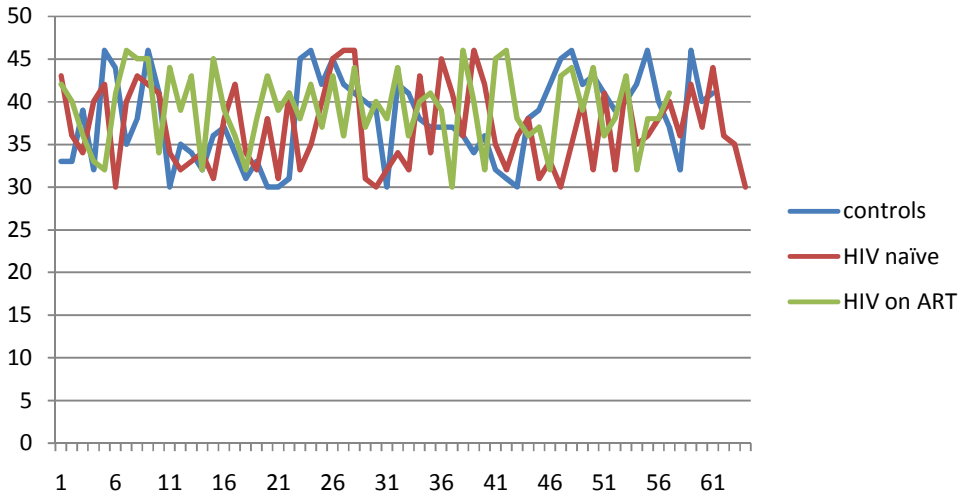


Table 2 **Age comparison between groups.**

Group	Mean age	Std. Deviation	p value
Control	38(30-46)	5.116	0.06
HIV without ART	37(30-46)	4.817	
HIV with ART	39.3(30-45)	4.702	
Total	38(30-46)	4.96	

Mean age among controls was 38 years with the standard deviation of 5.116, in HIV naive patients it was 37(SD: 4.817), and the mean age in HIV patients on ART was 39.3 (SD:4.702). Males were dominated in the sex distribution with 64.3%. There were no statistically significant difference between groups in the sex and age.

Figure 3 **Groups and sex distribution**

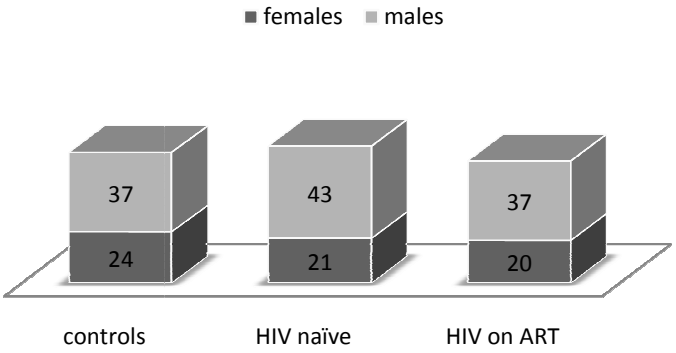


Table 3 **Comparing the groups in sex distribution.**

Group		Sex		Total	P value
		Male	Female		
Control	Count	37	24	61	0.743
	% within group	60.7%	39.3%	100%	
	% within sex	31.6%	36.9%	33.5%	
HIV naive	Count	43	21	64	
	% within group	67.2%	32.8%	100%	
	% within sex	36.8%	32.3%	35.2%	
HIV on ART	Count	37	20	57	
	% within group	64.9%	35.1%	100%	
	% within sex	31.6%	30.8%	31.3%	
Total	Count	117	65	182	
	% within group	64.3%	35.7%	100%	
	% within sex	100%	100%	100%	

Figure 4 **Distribution of smokers overall and within the groups.**

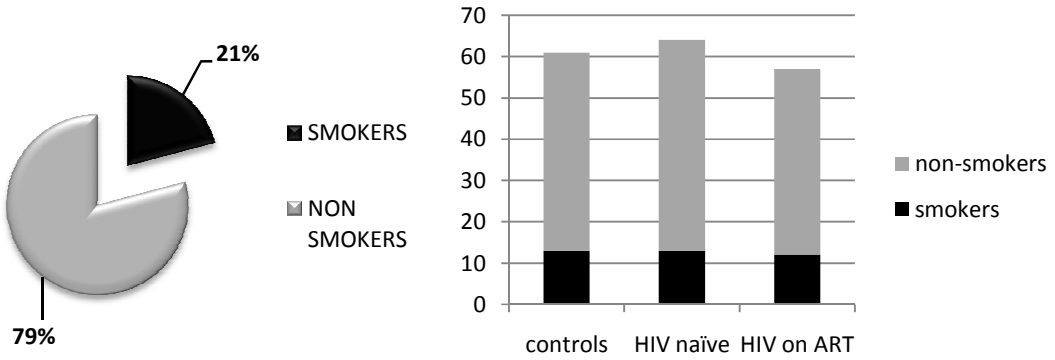


Table 4 **Comparing the smokers distribution between groups**

Group		Smoking		Total	P value
		Yes	No		
Control	Count	13	48	61	0.997
	% within group	21.3%	78.7%	100%	
	% within smoking	32.2%	33.3%	33.5%	
HIV naive	Count	13	51	64	
	% within group	20.3%	79.7%	100%	
	% within smoking	34.2%	35.4%	35.2%	
HIV on ART	Count	12	45	57	
	% within group	21.1%	78.9%	100%	
	% within smoking	31.6%	31.3%	31.3%	
Total	Count	38	144	182	
	% within group	20.9%	79.1%	100%	
	% within smoking	100%	100%	100%	

In the control group, 21.3% were smokers, group 2, and group 3 had smokers of 20.3%, 21.1%. P value was 0.997.

Figure 5 **BMI differences between groups.**

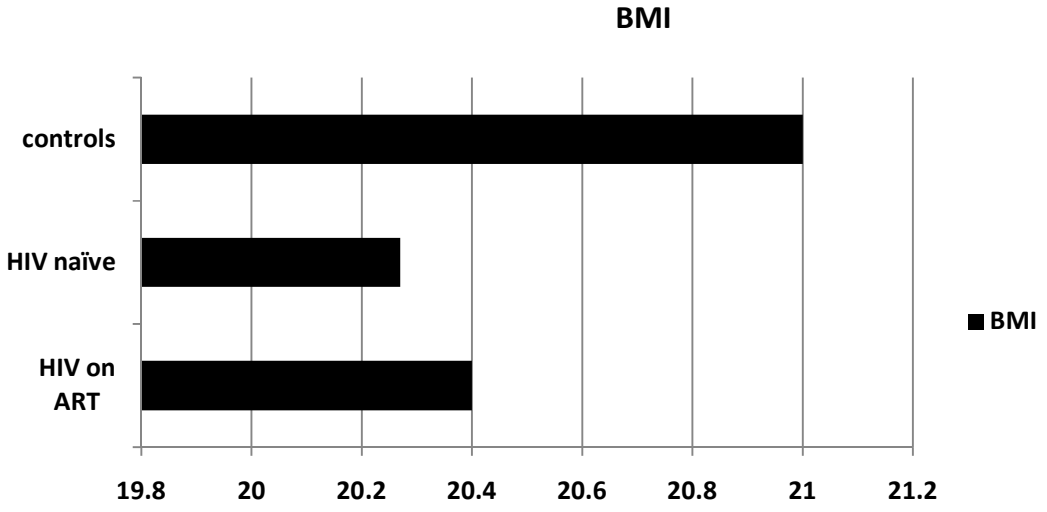


Table 5 **Comparison of the BMI between groups.**

Variable	Group	No. of individuals	Mean	Std. deviation	P value
BMI Index	Control	61	20.99175	1.430901	<0.001
	HIV without ART	64	20.27362	1.018532	
	HIV with ART	57	20.40400	0.845831	
	Total	182	20.55514	1.166456	

In HIV patients, average duration of disease was 40.1months (29-55months) with 39.6 months in HIV naives and 40.6 months in HIV patients on ART. The mean duration of ART treatment was 35.1 months (29-41months) in group 3. Mean BMI in HIV naive patients, was 20.27(SD: 1.02), and in HIV patients on ART, it was 20.4 (SD: 0.846). Controls had little bit higher BMI score (mean: 21; SD: 1.43), than HIV patients (group 2+3) (mean: 20.33; SD: 0.94). The difference was significant ($p<0.001$).

The mean Hb level in controls was 11.7gm/dl, for HIV patients it was 10.3gm/dl. When compared with controls (group 1), HIV patients (group 2+3) had significant differences in the haemoglobin levels ($p<0.001$).

Significant differences also noted in serum protein levels (controls: mean-7.24gm/dl; HIV positive-6.8gm/dl: $p<0.001$), serum albumin levels (controls:4.1gm/dl; HIV positive-3.8gm/dl: $p<0.001$).

Table 6 **Differences in the haemoglobin, protein, albumin levels between groups.**

Variables	Groups	No. of individuals	Values (gm/dl)	Std. deviation	Significance (p value)
Haemoglobin	Control	61	11.687	1.3239	<0.001
	HIV naive	64	10.163	1.2053	
	HIV on ART	57	10.475	1.2633	
	Total	182	10.771	1.4218	
Total Protein	Control	61	7.246	0.2884	<0.001
	HIV naive	64	6.795	0.4513	
	HIV on ART	57	6.954	0.3157	
	Total	182	6.996	0.4064	
Albumin	Control	61	4.113	0.2711	<0.001
	HIV naive	64	3.766	0.3591	
	HIV on ART	57	3.867	0.2552	
	Total	182	3.914	0.3335	

Differences In The HDL Levels Between Groups:

The mean serum HDL cholesterol level in controls was 51 mg/dl. In HIV naïve patients, it was 47.8 mg/dl, and in HIV patients with ART, it was 48 mg/dl. There were no statistically significant differences between the groups. No associations were found between the serum HDL levels and the CD4 count or duration of ART therapy. But there was a negative association found between the serum HDL cholesterol levels and the carotid intimal medial thickness levels. The Pearson correlation score was -0.387 with the 2-tailed significance of <0.001.

Figure 6 **Comparison of HDL cholesterol levels between groups.**

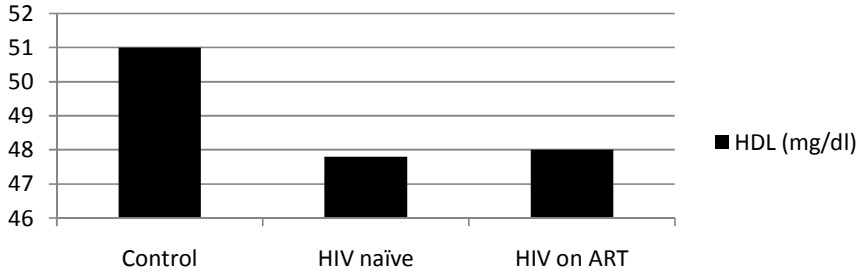


Table 7 **Comparing HDL levels among groups.**

Groups	Mean HDL (mg/dl)	Minimum	Maximum	Significance (p value)
Control	51	39	62	0.081
HIV naive	47.8	40	58	
HIV on ART	48	36	58	
Total	48.4	36	62	

Figure 7 **Differences in the triglyceride levels among groups.**

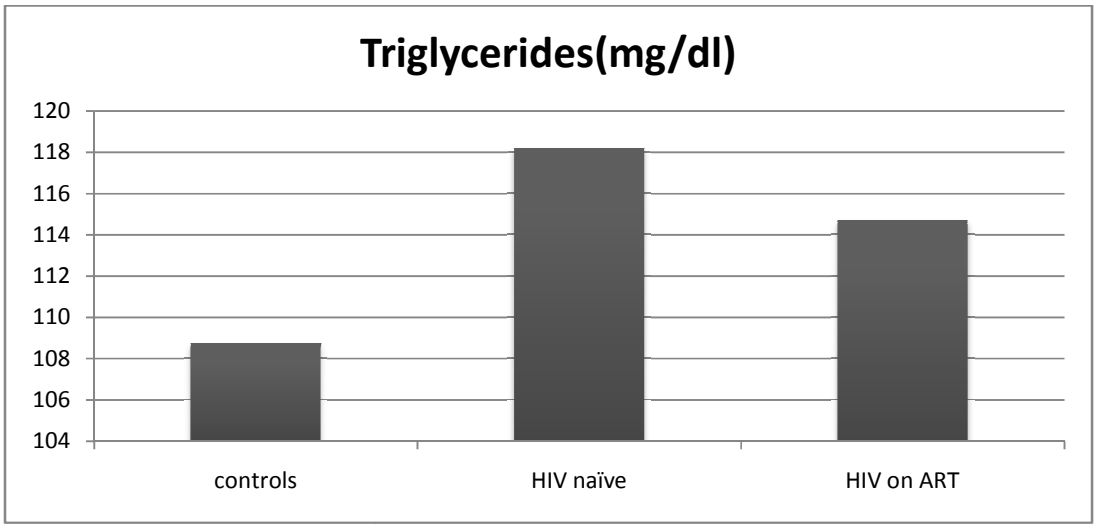


Table 8 **Comparison table for the triglyceride levels between groups.**

Variable	Group	No. of individuals	Mean (mg/dl)	Std. Deviation	Significance
Triglycerides	Control	61	108.74	11.897	<0.001
	HIV naive	64	118.17	13.389	
	HIV on ART	57	114.68	14.681	
	Total	182	113.92	13.842	

HIV patients had higher level of triglyceride levels when compared with controls. Among the HIV patients, when compared to HIV naives, patients on ART had lower level of triglycerides ($p<0.001$).

No correlation was found between the total cholesterol, and the IMT levels. In the waist circumference, controls had a mean of 66.9cm, HIV naive 65.75cm, and in HIV with ART 65.9cm: there were no significant differences between groups.

Table 9 **Comparing the differences between the LDL, total cholesterol, fasting blood sugar, waist circumference between groups.**

Variable	Group	Count	Mean	SD	Significance
Waist circumference	Control	61	66.89	5.648	.444
	HIV naive	64	65.75	4.458	
	HIV on ART	57	66.14	4.930	
W/H ratio	Control	61	.8361	.05315	.624
	HIV naive	64	.8404	.04949	
	HIV on ART	57	.8311	.05517	
FBS	Control	61	87.41	7.152	.548
	HIV naive	64	88.84	7.901	
	HIV on ART	57	87.70	8.073	
Total cholesterol	Control	61	168.44	14.627	.702
	HIV naive	64	169.44	13.015	
	HIV on ART	57	167.33	13.470	
LDL	Control	61	95.662	11.5722	.347
	HIV naive	64	97.991	10.4524	
	HIV on ART	57	98.046	8.4519	

Carotid IMT Measurements:

The mean maximal IMT (Carotid intimal medial thickness) was 0.726 mm for HIV-infected subjects, with standard deviation of 0.0189. For controls, the IMT was 0.686mm with standard deviation of 0.0208. Mean difference between HIV-infected vs controls was +0.04 mm (95%CI 0.0335 to 0.0461, p <0.0001).

Figure 8 **Picture showing the differences in the carotid IMT levels between the groups.**

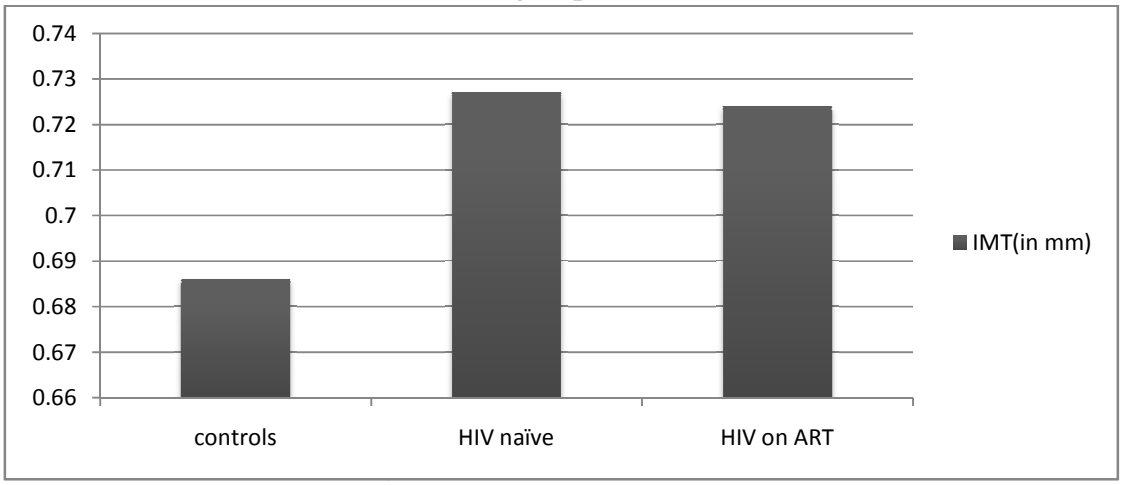


Table 10 **Comparing the carotid IMT values between the groups.**

	HIV status	No. of individuals	Mean	SD	P value
IMT	Control	61	0.68634	0.020857	<0.001
	HIV naive	64	0.72718	0.020801	
	HIV on ART	57	0.72431	0.016523	

The median IMT in group 1, group 2, and group 3 was 0.686, 0.727 and 0.724 mm, respectively.

Carotid Intimal Thickness Differences Between The Men And Women:

The mean maximal carotid intimal thickness in males was 0.724 mm with standard deviation of 0.0231 and in females 0.692mm with standard deviation of 0.0205. Mean difference between males and females was +0.032 mm (95%CI 0.0257 to 0.0391).

Figure 9 **Picture comparing the differences in the carotid IMT between males and females.**

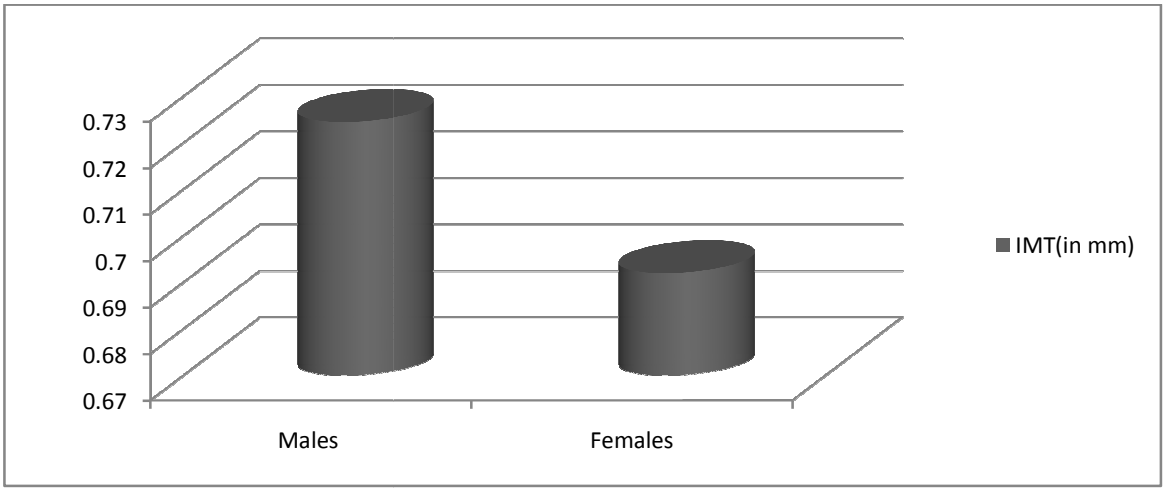
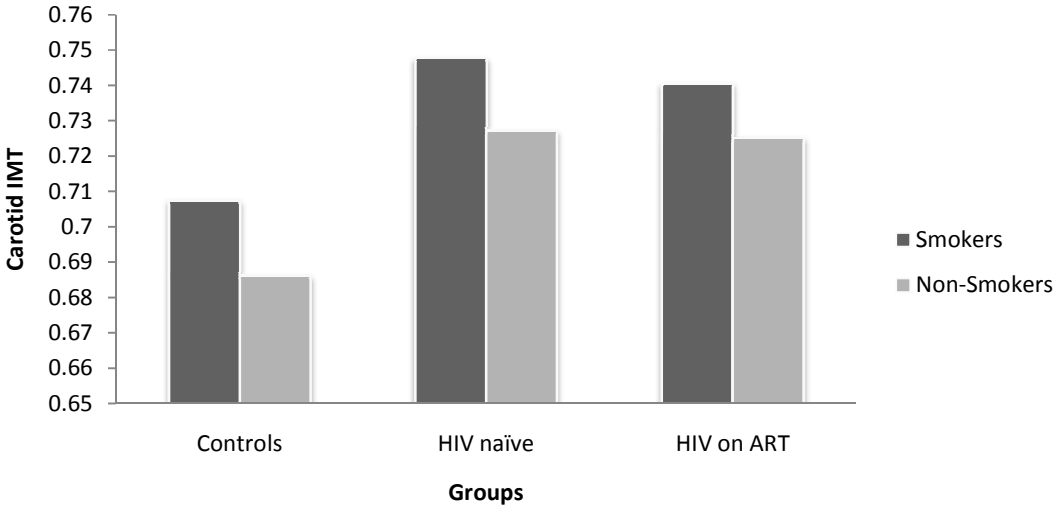


Table 11 **Comparison of carotid IMT between males and females. Males had higher IMT values which was highly significant.**

	Sex	No. Of individuals	Mean	SD	Std.error of mean	Significance (p value)
IMT	Males	117	0.724	0.023	0.0021	<0.001
	Females	65	0.692	0.020	0.0025	

Correlation Between Smoking And Carotid IMT:

Figure 10 **Picture comparing carotid IMT differences between smokers and non-smokers of each** group.



Compare to non-smokers, smokers had higher IMT values which was statistically significant. In the HIV positive individuals, the IMT values were higher among smokers.

Table 12 **Comparison of IMT values between smokers and non-smokers of each group, and between groups.**

Group	Smoking status	No. of individuals	Mean IMT (mm)	Significance (p value)
Controls	Smokers	13	0.7073	<0.001
	Non-smokers	48	0.686	
HIV naive	Smokers	13	0.7476	<0.001
	Non-smokers	51	0.7270	
HIV on ART	Smokers	12	0.7405	0.001
	Non-smokers	45	0.7250	

Groups And Atheromatous Plaque:

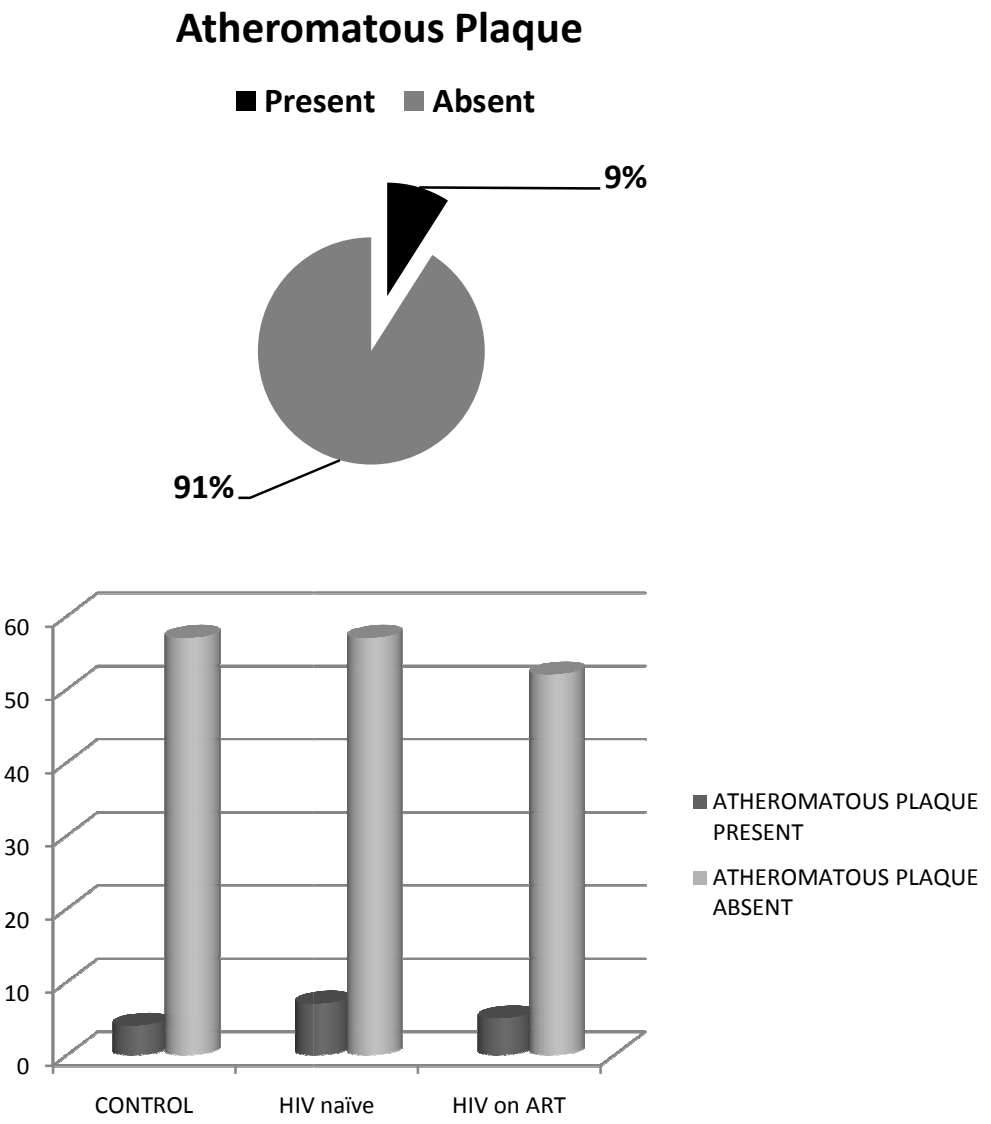
There were no significant differences in the distribution of Atheromatous plaques between the groups. The distribution of Atheromatous plaque were not increased in the HIV patients and it was also not significantly related ART therapy ($p > 0.05$). Smokers had higher prevalence of atheromatous plaques when compared to non-smokers.

Table 13 **Distribution of atheromatous plaque in the study population and in each group.**

Group		Atheromatous Plaque		Total	P value
		Yes	No		
Control	Count	4	57	61	0.688
	% within Group	6.6%	93.4%	100.0%	
	% within Atheromatous Plaque	25.0%	34.3%	33.5%	
HIV naive	Count	7	57	64	.685
	% within Group	10.9%	89.1%	100.0%	
	% within Atheromatous Plaque	43.8%	34.3%	35.2%	
HIV on ART	Count	5	52	57	.661
	% within Group	8.8%	91.2%	100.0%	
	% within Atheromatous Plaque	31.3%	31.3%	31.3%	
Total	Count	16	166	182	
	% within group	8.8%	91.2%	100.0%	

Distribution Of Atheromatous Plaque (Over All and Between Groups):

Figure 11 **Figures showing the distribution of plaques in the total study population and between groups. There were no significant differences between groups.**



Correlation And Regression Analysis:

The multivariate linear regression models were used to assess how well the Baseline covariates individually predict carotid IMT on the 182 subjects with Carotid IMT data. Regression analysis showed that the factors related to the carotid IMT were age, male sex, smoking, triglycerides, and HIV status (significance of <0.001 for all); and the standardized coefficients (beta) were orderly 0.152, 4.82, 0.169, 0.181, 0.624. This shows that the HIV status is independently associated with carotid IMT values. The impact was more than that of smoking.

Even after adjusting for traditional cardio vascular risk factors, the association between HIV and carotid IMT was still significant (P<0.001). Carotid IMT levels between the HIV naives and HIV patients on ART had a difference of 0.003mm which was not statistically significant. The correlation analysis showed that waist circumference (+0.27), triglycerides (+0.358), LDL levels (0.256), duration of the disease (+0.260), age (+0.318) are all directly correlated to the carotid IMT values (p<0.001).

The total CD4 counts were negatively correlated (-0.199) to the IMT values. Patients with lower CD4 counts had higher IMT values when compared to their matched ones. Likewise the HDL cholesterol levels were also had negative correlation (-0.387) with the IMT values (p<0.001).

Table 14 **Table showing the multivariate regression analysis of independent variables of carotid IMT predictors.**

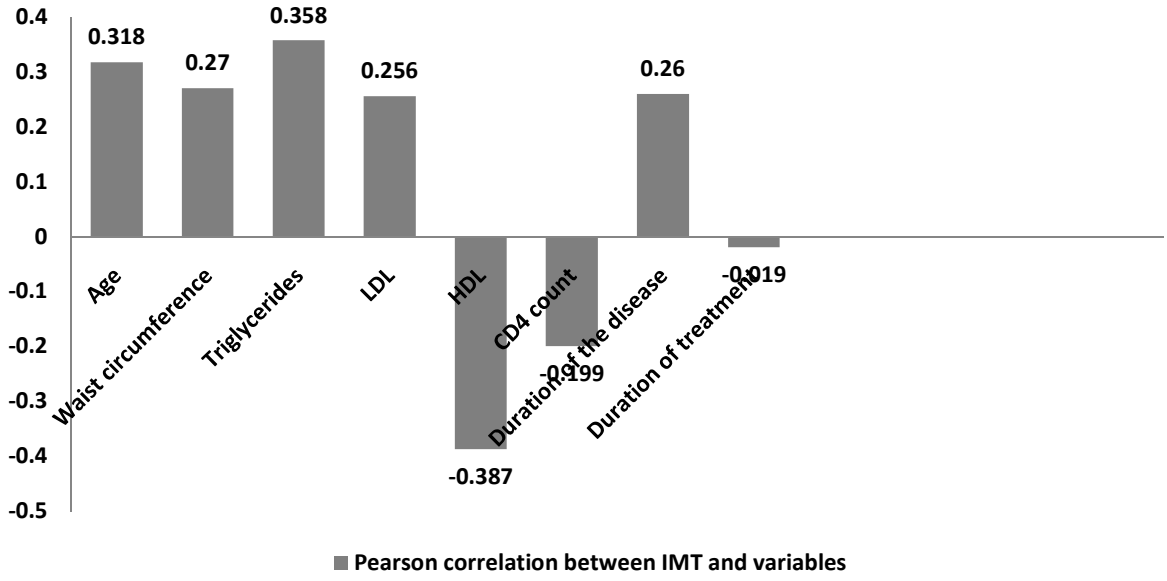
Independent variables	Unstandardized Coefficients		Standardized Coefficients (Beta)	Significance(p)
	IMT* values	Std. Error		
Constant	0.651	0.041		<0.001
Age in year	0.001	0.000	0.152	<0.001
Male Sex	0.027	0.005	0.482	<0.001
HIV status	0.036	0.003	0.624	<0.001
BMI Index	0.001	0.001	0.038	0.492
Waist circumference	0.000	0.000	0.040	0.442
W/H ratio	-0.015	0.046	-0.029	0.744
Smoking	0.011	0.002	0.169	<0.001
Tri Glycerides	0.000	0.000	0.181	<0.001
LDL	-1.519E-05	0.000	-0.006	0.888
HDL	0.000	0.000	0.063	0.104

*Carotid IMT: A Dependent Variable

Total cholesterol levels were not correlated well with the carotid IMT levels of study groups. There was no significance in the statistical analysis while comparing the total cholesterol level and the carotid IMTs of controls, and HIV patients with or without ART.

Correlation Between IMT And Variables:

Figure 12 **Picture showing the strength of correlation of variables with the carotid IMT.**



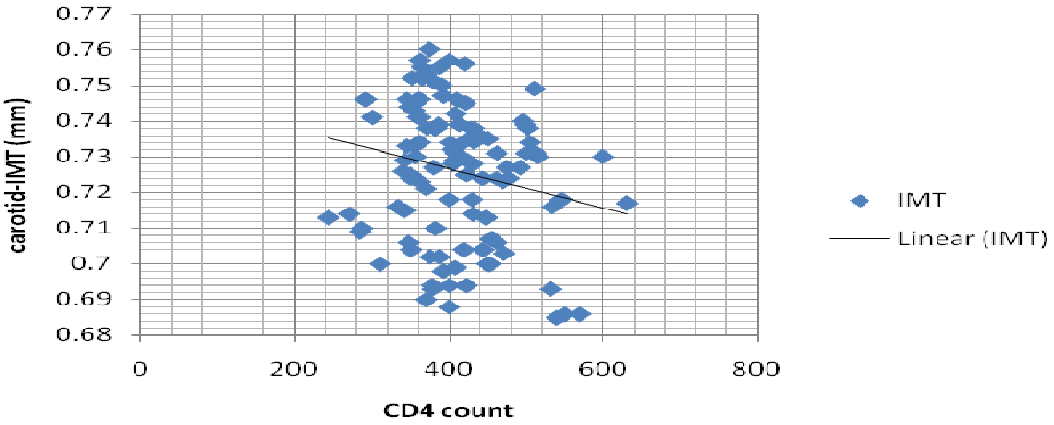
Positive Correlations:

1. Age (o.318)
2. Waist circumference (0.27)
3. Plasma triglyceride levels (0.358)
4. LDL cholesterol levels (0.256)
5. Duration of disease (0.26)

Negative Correlations:

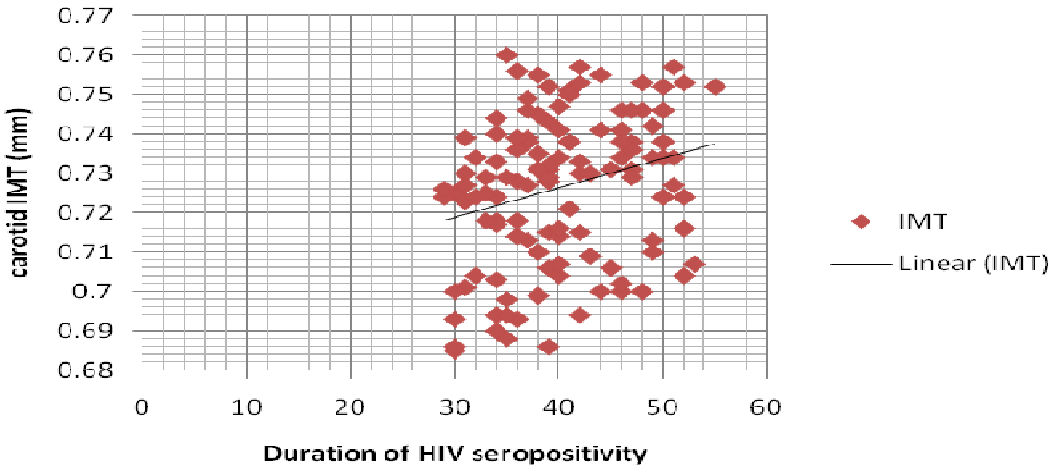
1. HDL cholesterol levels (-0.387)
2. CD4 counts (-0.199)
3. Duration of treatment (-0.019)

Figure 13 **Graph showing the correlation of carotid IMT with the CD4 count.**



CD4 count has inverse correlation with the carotid IMT values. As shown in the picture, the linear IMT values decreases when the CD4 count falls. This reflects the direct correlation of carotid IMT with the increasing viral load.

Figure 14 **Graph showing the correlation of carotid IMT with the duration of HIV seropositivity.**



Likewise carotid IMT is directly correlated duration of disease. As the duration of disease increases, the linear carotid IMT value goes up.

Table 15 **Pearson's correlation table for carotid IMT with independent variables.**

Independent variables	Number	Pearson correlation for IMT	Significance (p value; 2 tailed)
Waist circumference	182	0.270(**)	0.000
FBS	182	0.026	0.729
Total cholesterol	182	0.115	0.121
Tri Glycerides	182	0.358(**)	0.000
LDL	182	0.256(**)	0.000
HDL	182	-0.387(**)	0.000
CD4 count	121	-0.199(*)	.029
Duration of disease (months)	121	0.260(**)	0.004
Duration of Treatment (months)	57	-0.019	0.890
Age (years)	182	0.318(**)	0.000

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

CONCLUSION

- Even after adjustment for traditional CVD risk factors, HIV infected patients had higher subclinical atherosclerosis measured by IMT. The association of HIV infection with IMT was similar to that of traditional CVD risk factors, such as smoking. These effects could be due to chronic inflammation of the disease and the HIV virus per se.
- This accelerated progression could increase the incidence of CVD morbidities and mortalities among the HIV seropositives compared to HIV seronegatives.
- In our study NNRTIs and NRTIs based ART regimen not shown to have significant impact on subclinical atherosclerosis. But the correlations were on the negative side. This shows that the ART therapy may retard the progression of atherosclerosis in HIV patients.
- Monitoring for traditional risk factors, steps to modify risk factors such as smoking is very important in HIV seropositive individuals.
- CD4 counts are inversely related to the progression of atherosclerosis.
- Periodical monitoring of lipid abnormalities and efforts to control other risk factors are mandatory in the prevention of ART related cardiovascular complications.

- Our study encodes for the requirement of large volume, longitudinal follow-up studies to reveal the causal relationship of HIV effect and atherosclerosis.
- Use of lipid lowering drugs, the role of NNRTIs in the reduction of lipid abnormalities in HIV patients has to be further studied.